MOLECULAR DATA INDICATE COMPLEX INTRA- AND INTERCONTINENTAL DIFFERENTIATION OF AMERICAN *DRABA* (BRASSICACEAE)[1]

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ABSTRACT

The genus *Draba* includes about 350 species distributed primarily in the Northern Hemisphere, with some 80 species in South and Central America. Although species of *Draba* are well described morphologically, the existing sectional classification is highly controversial. American taxa exhibit enormous morphological differences even among species of the same section. We tested the hypothesis that variation accumulated during migration and differentiation of American *Draba*. The present phylogenetic study is based on analyses of the ITS (internal transcribed spacer) regions of the nuclear ribosomal DNA and the chloroplast *trn*L-intron and spacer sequences from 72 American *Draba* taxa and 6 European *Draba* species. Results suggest that some intrageneric groupings correspond primarily to phytogeography, and that only to a small degree do these findings agree with previous sectional classification. Differences between ITS- and *trn*L-derived phylogenies suggest extensive genetic contact may have existed between some of the groups or sections and that this disjunction between European and American *Draba* is demonstrated by ITS sequence data. Plastid DNA sequences suggest that the "European" plastome types may be more widely distributed among the American *Draba* species, perhaps through multiple transmissions of Eurasian chloroplast types into American *Draba*. Additional systematic analysis demonstrates that the genus *Erophila* has to be integrated into *Draba*. Analysis on the tribal level reveals the entire *Draba* complex to be close to European *Arabis* and *Aubrieta*. The data provide additional support for previous assumptions that the existing tribal classifications of the Brassicaceae are mostly artificial and that the segregation of *Draba* and *Arabis* into separate tribes or subtribes does not accurately reflect their phylogenetic relationship.

Key words: Brassicaceae, *Draba*, *Erophila*, molecular systematics, reticulate evolution.

The wide occurrence of polyploidy in vascular plants reflects its importance to plant speciation. The origin of polyploids and the mechanisms behind the establishment of newly evolved populations and taxa are among the challenging questions in plant sciences (Ramsey & Schemske, 1998; Thompson & Lumaret, 1992; Petit et al., 1999; Soltis & Soltis, 1999). New combinations of favorable genes may be more easily stabilized in polyploid taxa, and the permanent coexistence of favorable traits and characters from different parental lines may be effectively preserved as fixed heterozygosity (Soltis & Soltis, 1993). These speciation processes are integral to the genesis and maintenance of plant biodiversity. Recent studies of the overall genome structure of hybrid and polyploid taxa provide new insights about the dynamic nature of complete genomes either analyzed on the basis of artificial hybrids or by comparative mapping (Rieseberg et al., 1999; Rieseberg & Linder, 1999; Lagercrantz, 1998; Kowalski et al., 1994). In their comparative genome analysis of some Brassicaceae, Acarkan et al. (2000) showed that structural rearrangements occurred with a significantly higher frequency in polyploid *Brassica* L. than in diploid *Arabidopsis thaliana* (L.) Heynh. or *Capsella rubella* Reuter. This suggests that at the structural level a more dynamic nature of the genome might be sustained in polyploid taxa than in diploid ones, and this might be a remarkable source for new genetic variation upon which selective pressures can then act.

Draba L. is the largest genus of the Brassicaceae and includes ca. 350 species, or about 10% of the family total (Al-Shehbaz, 1987). The genus is distributed primarily in the Northern Hemisphere, especially in the subarctic to arctic regions and alpine or mountainous portions of the temperate regions. Nearly half of the taxa are found in the

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New World and about 80 species are distributed in Central and South America (Al-Shehbaz, 1987). Schulz (1927, 1936) divided the genus into 17 sections (Table 1), but Fernald (1934) criticized this artificial sectional classification largely on its misleading and impractical keys. Further, Tolmachev (1939) totally ignored Schulz's sections and recognized 29 series for the 91 species occurring in the former Soviet Union. No subsequent botanists have attempted to subdivide *Draba* into infrageneric taxa. For European *Draba*, most authors have followed Walters (1964) who correctly used section *Draba* instead of Schulz's section *Leucodraba* DC., because section *Draba* includes the generic type.

A few case studies have investigated the phylogenetic relationships among some Arctic Scandinavian (Brochmann et al., 1992a–d, 1993) and alpine European (Widmer & Baltisberger, 1999a, b) species of *Draba*. Some general aspects of these studies have been previously outlined (Brochmann, 1992) and can be summarized as: (1) allopolyploidy is common, with a polyphyletic and polytopic origin for some taxa; (2) gene flow across different ploidy levels is possible and probably occurs in natural populations; (3) complex evolutionary phylogenetic networks have been demonstrated for section *Draba* and some species of section *Chrysodraba*; (4) repeated migration and colonization contribute to complex distribution patterns; and (5) not unexpectedly, items 1 through 4 are substantiated by molecular studies with complex patterns of intra- and interspecific variation observed (Brochmann et al., 1992a–d; Widmer & Baltisberger, 1999a, 1999b). Support also exists at morphological, cytological, and ecological levels (Brochmann, 1993; Brochmann et al., 1993).

Isozyme electrophoresis and DNA analysis of the nuclear and plastid genome have greatly increased the possibility of detecting and distinguishing allo- and autopolyploids, to trace paternal and maternal genome lineages, and to document hybridization, introgression, and reticulate evolution within polyploid complexes. Several polyploid complexes in the Brassicaceae have been characterized, including those of the genera *Microthlaspi* F. K. Meyer (Koch et al., 1998b; Koch & Hurka, 1999), *Draba* (Brochmann & Elven, 1992; Brochmann, 1993; Brochmann et al., 1992a–d, 1993), *Cochlearia* L. (Koch et al., 1998a, 1999b), *Yinshania* Y. C. Ma & Y. Z. Zhao (Koch & Al-Shehbaz, 2000), *Cardamine* L. (Franzke et al., 1998; Urbanska et al., 1997), *Biscutella* L. (König, 1998), *Brassica* L. and related genera (Anderson & Warwick, 1999), and *Nasturtium* R. Br. (Bleeker et al., 2000).

In order to gain a better understanding of the phylogenetic relationships within a large polyploid complex, we examined the sequence variation from the internal transcribed spacer regions (ITS1 and ITS2) of nrDNA for American species of *Draba* (Baldwin et al., 1995; Campbell et al., 1995), the cp *trn*L intron (Taberlet et al., 1991; Gielly & Taberlet, 1994; van Ham et al., 1994; Koch & Al-Shehbaz, 2000), and the cp *trn*L-*trn*F spacer (Taberlet et al., 1991). The derived molecular phylogenies were then compared with traditional concepts based on morphological data.

Materials and Methods

Plant material, DNA extraction, PCR-amplification, and sequencing

Leaf material for DNA extraction was obtained from herbarium specimens (Table 1). DNA was isolated from 25–50 mg of dried leaf material using the NucleonPhytoPure Kit (Amersham Lifescience) following the instructions of the supplier. DNA was stored in 10 mM Tris-EDTA buffer pH 8.0 at −20°C.

Double-stranded DNA of the complete ITS region, including the 5.8 S rDNA region, was amplified by 35 cycles of symmetric PCR using ITS primers initially designed by White et al. (1990) and modified by Mummenhoff et al. (1997a) for ITS4. The PCR profile used to amplify the ITS region followed the following profile: a hot start with 5 min. at 94°C, and 35 cycles of amplification (1 min. 94°C, 45 sec. 38°C, 45 sec. 72°C), final elongation step for 10 min. at 72°C, and storage at 4°C. The 18F primer (5′-GGAAGGAGAAGTCGTAACAAGG-3′) is located at the 3′-end of the 18 S rDNA gene and primer 25R (5′-TCCTCCGCTTATTGATATGC-3′) is located at the 5′-end of the 25 S rDNA. PCR products were purified using the Boehringer PCR product purification kit (Roche Molecular Biochemicals). PCR products spanned the entire ITS1, 5.8 S rDNA, and ITS2 region and were cycle-sequenced directly without cloning using the Taq DyeDeoxy Terminator Cycle Sequencing Kit (ABI Applied Biosystems, Inc.) and the 18F and 25R primers. Products of the cycle sequencing reactions were run on an ABI 377XL automated sequencer.

The *trn*L (UAA) intron was amplified and sequenced by using the universal primers "c" (B49317, 5′-CGAAATCGGTAGACGCTACG-3′) located at the 3′-end of the *trn*L(UAA)5′-exon and "d" (A49855, 5′-GGGGATAGAGGGACTTGAAC-3′) located at the 5′-end of the *trn*L(UAA)3′-exon (Taberlet et al., 1991). The PCR profile used to amplify the *trn*L intron followed Koch and Al-Sheh-

Table 1. Accession data of the material under study. Additional explanation is indicated on the table itself.

Taxon	Country, collector (herbarium acronym)	Acc. no.	Sectional classification according to Schulz (1927): see Table 2	ITS1, 5.8S rDNA, and ITS2	*trn*L intron	*trn*L spacer
Cusickiella douglasii (A. Gray) Rollins	U.S.A., *Tiehm & Williams 7998* (MO)	78		AF146515	AF146959	AF146962
C. quadricostata (Rollins) Rollins	U.S.A., *Tiehm & Williams 7929* (MO)	77		AF146514	AF146960	AF146961
Draba aizoides L.	Germany, *Neuffer s.n.* (OSBU #3818)	103	*Aizopsis*	AF146511	AF146957	AF146964
D. aizoides L.	Switzerland, Widmer & Baltisberger, 1999b	107	*Aizopsis*	AF120721	AF120727	
D. aizoides L.	Switzerland, Widmer & Baltisberger, 1999b	108	*Aizopsis*		AF120730	
D. aizoides L.	Switzerland, Widmer & Baltisberger, 1999b	109	*Aizopsis*		AF120731	
D. aizoides L.	Austria, *Neuffer s.n.* (OSBU#5199)	104	*Aizopsis*	AF146512	AF146958	AF146963
D. alyssoides HBK	Ecuador, *Sklenar & Kostechova 162* (MO)	23		AF146456	AF146904	AF147017
D. araboides Wedd.	Peru, *Tovar 959* (US)	84	*Tomostima*	AF146505	AF146952	AF146969
D. aureola	U.S.A., *Hurka 3189* (OSBU)	101	*Phyllodraba*	AF146509	AF146956	AF146965
D. barclayana Al-Shehbaz	Colombia, *Barclay & Juajibioy 7398* (GH)	11		AF146449	AF146898	AF147023
D. bellardii S. F. Blake	Venezuela, *Ruiz-Terán & López-Figueiras 10469* (MO)	7	*Chamaegongyle*	AF146446		
D. boyacana Al-Shehbaz	Colombia, *Grubb et al. 303* (K)	79		AF146500	AF146947	AF146974
D. carinthiaca Hoppe	Switzerland, Widmer & Baltisberger, 1999b	105	*Leucodraba*		AF120737	
D. chionophila S. F. Blake	Venezuela, *Berry 4273* (MO)	8	*Chamaegongyle*	AF146447	AF146897	AF147024
D. confertifolia Turcz.	Ecuador, *Spruce 5796* (MO)	35	*Calodraba*	AF146463	AF146910	AF147011
D. crassifolia Graham	U.S.A., *Stein et al. 1881* (MO)	59	*Drabella*	AF146482	AF146930	AF146991
D. cruciata Payson	U.S.A., *Sharsmith 3338* (MO)	60	*Chrysodraba*	AF146483	AF146931	AF146990
D. cryophila Cuatrec.	Colombia, *Cuatrecasas & Romero 24611* (US)	5		AF146444	AF146895	AF147026
D. cryptantha Hook. f.	Peru, *Smith et al. 10673* (MO)	28	*Tylodraba*	AF146457		
D. cuatrecasana Rangel & Santana	Colombia, *Cleef 7668* (US)	1		AF146441	AF146892	AF147029
D. cuneifolia Nutt. ex Torr. & A. Gray	U.S.A., *Rollins & Rollins 8747* (MO)	61	*Tomostima*	AF146484	AF146932	AF146989
D. densifolia Nutt.	U.S.A., *Hurka 2837* (OSBU)	102	*Chrysodraba*	AF146510		
D. densifolia Nutt. ex Torr. & A. Gray	U.S.A., *Rollins & Rollins 78228* (MO)	62	*Chrysodraba*	AF146485	AF146933	AF146988
D. depressa Hook. f.	Ecuador, *Sklenar & Kostechova 1269* (MO)	30	*Tylodraba*	AF146458	AF146905	AF147016
D. dubia Suter	Switzerland, Widmer & Baltisberger, 1999b	114	*Leucodraba*	AF120722	AF120738	
D. extensa Wedd.	Ecuador, *Asplund 8595* (US)	85	*Tylodraba*	AF146506	AF146953	AF146968
D. farsetioides Linden & Planch.	Venezuela, *RuizTerán & López-Figueiras 1064* (MO)	31	*Calodraba*	AF146459	AF146906	AF147015
D. funckiana Linden & Planch.	Venezuela, *Gines 4706* (US)	32	*Chamaegongyle*	AF146460	AF146907	AF147014
D. funckiana Linden & Planch.	Venezuela, *Gines 4706* (US)	87	*Chamaegongyle*	AF146508	AF146955	AF146966
D. funiculosa Hook. f.	Argentina, *Pisano & Garaventa 2736* (MO)	33	*Linodraba*	AF146461	AF146908	AF147013

Table 1. Continued.

Taxon	Country, collector (herbarium acronym)	Acc. no.	Sectional classification according to Schulz (1927); see Table 2	ITS1, 5.8S rDNA, and ITS2	*trn*L intron	*trn*L spacer
D. gilliesii Hook. & Arn.	Chile, *Taylor & Taylor 108848* (MO)	34	*Calodraba*	AF146462	AF146909	AF147012
D. glabella Pursh	U.S.A., *Hodgdon 8922* (MO)	63	*Leucodraba*	AF146486	AF146934	AF146987
D. hallii Hook. f.	Ecuador, *Humbles 6330* (MO)	36	*Calodraba*	AF146464	AF146911	AF147010
D. hammenii Cuatrec & Cleef	Colombia, *Cleef 9070* (US)	2		AF164442	AF146893	AF147028
D. helleriana Greene	Mexico, *Sharp 45753* (MO)	54	*Phyllodraba*	AF146478	AF146925	AF146996
D. hemsleyana O. E. Schulz	Ecuador, *Ramsay & Merrow-Smith 24* (MO)	83	*Calodraba*	AF146504	AF146951	AF146970
D. hidalgensis Calderón	Mexico, *Rzedowski 26804* (MO)	55		AF146479	AF146926	AF146995
D. hitchcockii Rollins	U.S.A., *Rollins et al. 83263* (MO)	64		AF146487	AF146935	AF146986
D. incerta Payson	U.S.A., *Rollins et al. 83323* (MO)	65	*Leucodraba*	AF146488	AF146936	AF146985
D. jaegeri Munz & Johnson	U.S.A., *Clokey 7949* (MO)	66		AF146489	AF146937	AF146984
D. jorullensis HBK	Mexico, *Weaver 2126* (MO)	56	*Adenodraba*		AF146927	AF146994
D. jorullensis HBK	Mexico, *Breedlove 26720* (MO)	57	*Adenodraba*	AF146480	AF146928	AF146993
D. ladina Br.-Bl.	Switzerland, Widmer & Baltisberger, 1999b	115	*Leucodraba*	AF120723	AF120740	
D. ladina Br.-Bl.	Switzerland, Widmer & Baltisberger, 1999b	116	*Leucobrada*	AF120724		
D. lapaziana Al-Shehbaz	Bolivia, *Solomon 13219* (MO)	21		AF146455	AF146903	AF147018
D. lindenii (Hook.) Planch.	Venezuela, *Berry 4029* (MO)	38	*Doliostylis*	AF146465	AF146912	AF147009
D. litamo Uribe	Colombia, *Grupp et al. 833* (MO)	39		AF146466		
D. macleanii Hook. f.	Peru, *Smith et al. 10674* (MO)	40	*Rhabdodraba*	AF146467	AF146913	AF147008
D. magellanica Lam.	Argentina, *Pisano & Garaventa 2717* (MO)	41	*Leucodraba (Holarges)*		AF146914	AF147007
D. matthioloides O. E. Schulz	Peru, *Holt 191* (K)	82	*Calodraba*	AF146503	AF146950	AF146971
D. mogollonica O. E. Schulz	Peru, *Holt 191* (K)	67	*Phyllodraba*	AF146490	AF146938	AF146983
D. nivicola Rose	Mexico, *Zola et al. 72* (MO)	58	*Adenodraba*	AF146481	AF146929	AF146992
D. oligosperma Hook.	U.S.A., *Rollins & Rollins 79233* (MO)	68	*Chrysodraba*	AF146491	AF146939	AF146982
D. paysonii Macbride	U.S.A., *Rollins et al. 83306* (MO)	71	*Chrysodraba*	AF146494	AF146941	AF146980
D. pennell-hazenii O. E. Schulz	Colombia, *Forero et al. 3634* (MO)	3	*Chamaegongyle*	AF146443	AF146894	AF147027
D. peruviana (DC.) O. E. Schulz	Peru, *Macbride 4440* (MO)	43	*Calodraba*	AF146468	AF146915	AF147006
D. pickerengii A. Gray	Peru, *Solomon 3329* (MO)	44	*Rhabdodraba*	AF146469	AF146916	AF147005
D. platycarpa Torr. & A. Gray	U.S.A., *Correll & Little 27171* (MO)	69	*Tomostima*	AF146492	AF146940	AF146981
D. pseudocheiranthoides Al-Shehbaz	Colombia, *Barclay & Juajibioy 6733* (MO)	12		AF146450		
D. pterosperma Payson	U.S.A., *Hitchcock & Martin 5317* (MO)	70	*Chrysodraba*	AF146493		
D. pulvinata Turcz. subsp. *pulvinata*	Venezuela, *Ewan 16951* (K)	80	*Doliostylis*	AF146501	AF146948	AF146973

Table 1. Continued.

Taxon	Country, collector (herbarium acronym)	Acc. no.	Sectional classification according to Schulz (1927); see Table 2	ITS1, 5.8S rDNA, and ITS2	*trn*L intron	*trn*L spacer
D. pulvinata Turcz. subsp. *berryi* Al-Shehbaz	Venezuela, *Berry 4030* (MO)	18	*Doliostylis*	AF146454	AF146902	AF147019
D. pusilla F. Philippi	Chile, *Ruthsatz 7026* (MO)	45	*Drabella*	AF146470	AF146917	AF147004
D. pycnophylla Turcz.	Ecuador, *Luteyn et al. 8910* (MO)	46	*Calodraba*	AF146471	AF146918	AF147003
D. ramosissima Desvaux	U.S.A., *Kral 49698* (MO)	76	*Phyllodraba*	AF146499	AF146946	AF146975
D. reptans (Lam.) Fernald	U.S.A., *Muehlenbach 3498* (MO)	72	*Tomostima*	AF146495	AF146942	AF146979
D. ritacuvana Al-Shehbaz	Colombia, *Barclay & Juajibioy 7323-A* (GH)	13		AF146451	AF146899	AF147022
D. rositae Santana & Rangel	Colombia, *Rangel 3105* (GH)	14		AF146452	AF146900	AF147021
D. schusteri O. E. Schulz	Peru, *Weberbauer 6033* (GH)	15	*Rhabdodraba*	AF146453	AF146901	AF147020
D. scopulorum Wedd.	Bolivia, *Solomon 5023* (MO)	47	*Rhabdodraba*	AF146472	AF146919	AF147002
D. sericea Santana & Rangel	Colombia, *Cleef 5261* (MO)	48		AF146473	AF146920	AF147001
D. solitaria O. E. Schulz	Peru, *Weberbauer 7235* (US)	6	*Calodraba*	AF146445	AF146896	AF147025
D. soratensis Wedd.	Bolivia, *Solomon 13136* (MO)	49	*Rhabdodraba*	AF146474	AF146921	AF147000
D. splendens Gilg	Ecuador, *Ollgard & Balslev* 10080 (MO)	50	*Calodraba*	AF146475	AF146922	AF146999
D. spruceana Wedd.	Ecuador, *Spruce 5766* (GH)	9	*Adenodraba*	AF146448		
D. standleyi Macbride & Payson	U.S.A., *Goodman & Hitchcock 1168* (MO)	74		AF146497	AF146944	AF146977
D. streptocarpa A. Gray	U.S.A., *Park 86* (MO)	73	*Phyllodraba*	AF146496	AF146943	AF146978
D. stylaris Koch	Widmer & Baltisberger, 1999b	106	*Leucodraba*		AF120736	
D. stylosa Turcz.	Ecuador, *Jameson 150* (K)	81		AF146502	AF146949	AF146972
D. subumbellata Rollins & Price	U.S.A., *Morefield & Ross 4712* (MO)	75		AF146498	AF146945	AF146976
D. tomentosa Clairv.	Switzerland, Widmer & Baltisberger, 1999b	112	*Leucodraba*	AF120725	AF120741	
D. tomentosa Clairv.	Switzerland, Widmer & Baltisberger, 1999b	113	*Leucodraba*	AF120726		
D. tucumanensis O. E. Schulz	Argentina, *Jörgensen 1574* (MO)	51		AF146476	AF146923	AF146998
D. violacea HBK	Ecuador, *Jameson s.n.* (US#534920)	86	*Calodraba*	AF146507	AF146954	AF146967
D. wurdackii Al-Shehbaz	Peru, *Sánchez Vega 4149* (MO)	52		AF146477	AF146924	AF146997
Erophila spathulata A. F. Láng	Austria, *Englisch 2655/98*	111		AF377952		
E. verna (L.) Chev.	Austria, *Englisch 2711/98*	110		AF377951		

baz (2000): a hot start with 5 min. at 94°C, and 35 cycles of amplification (1 min. 94°C, 45 sec. 50°C, 45 sec. 72°C), a final elongation step for 10 min. at 72°C, and storage at 4°C. PCR products were purified and cycle-sequenced as described for ITS analysis using the amplification primer c and d. The *trn*L(UAA)-*trn*F(GAA) intergenic spacer was amplified using the following primers: the universal primer "e" designed by Taberlet et al. (1991) (5′-GGTTCAAGTCCCTCTATCCC-3′) and a newly designed primer *trn*F-IGS-rev (5′-AGGATTTTCAGTCCTCTGCTC-3′). Amplification, purification, and sequencing were performed as described for the *trn*L intron.

DATA ANALYSIS

Outgroup selection. The genus *Draba* has been variously placed into the tribes Alysseae or Drabeae (Hayek, 1911; Schulz, 1936; Janchen, 1942; Al-Shehbaz, 1987). However, as pointed out by Al-Shehbaz (1987), the Alysseae are a poorly defined tribe with about 40 genera (15 monotypic) and some 650 species distributed primarily in the Irano-Turanian and Mediterranean regions. Recent molecular studies (Price et al., 1994; Koch et al., 1999a, 2000, 1999b) clearly indicate that nearly all tribal subdivisions of the Brassicaceae are highly artificial. Therefore, we used the aligned ITS data set from Koch et al. (1999a) in which tribal structures were analyzed, and taxa from the tribes Lepidieae, Sisymbrieae, and Arabideae were considered to infer relative phylogenetic position of taxa under investigation. We added sequence data from *Erophila verna* (L.) Chev., *D. araboides* Wedd., *D. aizoides* L., and *Cusickiella quadricostata* (Rollins) Rollins (accession nos. 110, 84, 103, and 77, respectively, Table 1) to the alignment described by Koch et al. (1999a). We included *C. quadricostata* because it was originally described in *Draba*, and only recently it and *D. douglassii* A. Gray were transferred to the newly established *Cusickiella* Rollins (Rollins, 1988). Data analysis, distance and parsimony methods, were performed as described in Koch et al. (1999a) to get comparable results to this analysis. The results were used to choose an appropriate outgroup. Our analysis herein demonstrated that the European *Arabis* L. and *Aubrieta deltoidea* (L.) DC. are closer to *Draba* than *Cusickiella* is. Therefore, European *Arabis* (*A. alpina*, *A. bryoides*, *A. pumila*, *A. blepharophylla*) and *Aubrieta deltoidea* were selected as outgroups for the further ITS study of *Draba*. *Cusickiella* was excluded from all further analysis.

ITS data. DNA sequences were aligned by hand. In addition to sequences from American *Draba*, we included 6 ITS sequences from European *Draba* from Widmer and Baltisberger (1999a, Table 1) and 6 sequences from *Arabis/Aubrieta* serving as outgroups (Koch et al., 1999a), resulting in a total matrix of 80 ITS sequences. Distance analyses were performed with Kimura-2-parameter distances using PHYLIP software package version 3.57c (Felsenstein, 1995). The neighbor-joining algorithm was used for tree construction. Bootstrap analysis (Felsenstein, 1985) was performed using 1000 replicates. For all analyses gaps were coded as missing characters. The parsimony analysis began with a subset of taxa comprising representatives from all putative clades included in the overall analysis. This analysis was performed to demonstrate relative branch length and to estimate additional confidence parameters. The second data matrix used was identical in alignment to the first data matrix. Parsimony analysis was performed with unordered Fitch parsimony using PAUP* 4.0b2 (Swofford, 1999). The branch-and-bound algorithm was used to find maximally parsimonious trees. Bootstrap analysis (Felsenstein, 1985) was performed using 1000 replicates with the bootstrap search algorithm. Decay analysis (Bremer, 1988) was performed in addition to the bootstrap approach, in order to assess the confidence that could be placed in the monophyly of clades. Decay indices (DI) were estimated according to Baum et al. (1994). For all parsimony analyses gaps were coded as missing characters.

trnL intron and spacer data. DNA sequences were aligned by hand using the boundaries of coding regions as anchor points with which to begin the alignments. We included 8 *trn*L intron and spacer sequences from European *Draba* (Widmer & Baltisberger, 1999b, Table 1) and 67 American *Draba* for a total of 75 *trn*L sequences. *Draba cuneifolia* (acc. no. 61), *D. platycarpa* (acc. no. 69), *D. reptans* (acc. no. 72), and *D. araboides* (acc. no. 84), which are only distantly related to remaining ingroup taxa (results from the ITS analysis), were used as outgroups. The ancestral position of these four taxa with respect to the remaining *Draba* analyzed herein was also confirmed by an analysis using *trn*L intron and *trn*L-*trn*F spacer regions from *Arabis alpina* L. and *Aubrieta deltoidea* L. (GenBank accession numbers AY034180 and AY034181, respectively). Distance analyses were performed for the total data set as described for the ITS data. In order to obtain a relative support for branching patterns of representatives from all putative clades, a subset of *Draba* taxa was investigated with Fitch parsimony as described for ITS analysis. For this analysis gaps were coded as additional binary (0/1)

characters. All gaps differing in length and position were coded as separate binary characters. The distribution of gaps and its coding can be viewed at http://homepage.boku.ac.at/koch/.

Missing data. Amplification failed to yield ITS data for *Draba magellanica* (acc. no. 41) and *D. jorullensis* (acc. no. 56), and *trn*L intron and spacer data was missing for *D. bellardii* (acc. no. 7), *D. spruceana* (acc. no. 9), *D. pseudocheiranthoides* (acc. no. 12), *D. cryptantha* (acc. no. 28), *D. litamo* (acc. no. 39), *D. pterosperma* (acc. no. 70), and *D. densifolia* (acc. no. 102). For *Draba carinthiaca* (acc. no. 105) and *D. stylaris* (acc. no. 106) only *trn*L intron and spacer data were available, and for the two *Erophila* species no plastidic data were available. Data matrices are available upon request or can be viewed at http://homepage.boku.ac.at/koch/.

Results

Outgroup selection and tribal relationships

Our reexamination and analysis combining the ITS data matrix (a sampling of 36 ITS sequences from species across Arabideae, but excluding *Draba*) from Koch et al. (1999a) with sequences added from *Erophila verna*, *Draba araboides*, *D. aizoides*, and *Cusickiella quadricostata* (in capital letters, Fig. 1) resulted in trees identical in topology to the phylogenetic hypothesis presented in that paper. The data matrix of 603 bp contained 342 invariable characters. From the remaining 261 variable nucleotide positions 163 sites were potentially informative. Fitch parsimony analysis resulted in 12 most parsimonious trees with a length of 612 steps, a consistency index (CI) of 48.9% (autapomorphies excluded), and a retention index (RI) of 71.8%. The strict consensus tree (not shown) out of these 12 most parsimonious trees is identical (except for *Erophila verna*, *Draba aizoides* and *D. araboides*, and *Cusickiella quadricostata* that are added herein) to the previously published phylogenetic network (Koch et al., 1999a). The distance tree is identical to that presented by Koch et al. (1999a) (Fig. 1). *Draba* is closely related to the European *Arabis* L. and *Aubrieta* Adans., and it is positioned within an "Arabideae core group" (Fig. 1). The genus *Cusickiella*, which is endemic to the western United States, was resolved with North American *Halimolobus* Tausch and North American *Arabis*. As shown by Koch et al. (1999a), the North American *Arabis*, excluding the *A. blepharophylla* Hook. & Arn. group and a few other species, are better treated in the genus *Boechera* A. Löve & D. Löve (Löve & Löve, 1975). For subsequent ITS analysis of all *Draba* taxa, European *Arabis* and *Aubrieta* were used as outgroups, and *Cusickiella* has been excluded from all subsequent analysis.

ITS data

The analysis of phylogenetic relationships among ingroup taxa comprises 80 ITS sequences from *Draba* and *Erophila* of which 74 sequences are novel. Six ITS sequences from *Arabis* and *Aubrieta* were used to root phylogenetic trees as outlined above. The total length of the aligned data matrix is 626 bp. From 283 bp within the ITS1 region, 109 sites were variable (including 37 autapomorphic changes); from 166 bp within the 5.8 S rDNA, 11 sites were variable (all autapomorphic changes); and from 177 bp within the ITS2 region, 20 sites were variable (all autapomorphic changes). This results in a total of 68 potentially informative sites. Uncorrected pairwise sequence distances ranged up to 10% within outgroup taxa (59 nucleotide positions), up to 9.3% within ingroup taxa (57 nucleotide positions), and up to 12.3% among the whole data set. Argentinian *Draba funiculosa* Hook. f. (acc. no. 33) was excluded from these calculations because of an unusual ITS type that differed markedly from the remaining *Draba* taxa (13.1 to 16.6% sequence divergence or 82 to 104 nucleotide positions). However, in the phylogenetic analysis *D. funiculosa* clustered among European *Draba* (Fig. 2). The whole alignment required 44 gap positions of 1 nucleotide in length, and 2 gap positions required 4 (*Arabis alpina*, *A. blepharophylla*) and 14 (*A. alpina* L., *Aubrieta deltoidea*) base pairs, respectively. Identical ITS sequences were detected in *D. lindenii* and *D. pulvinata* subsp. *pulvinata* (acc. nos. 38 and 80, respectively) and in *D. sericea* (acc. no. 48), *D. splendens* (acc. no. 50), *D. hemsleyana* (acc. no. 83), and *D. extensa* (acc. no. 85). The results of the distance analysis are shown in Figure 2. Six major clades (I–VI as indicated on Fig. 2) could be distinguished among the genus *Draba* with relatively robust support for clades I–III (Fig. 2).

We also indicated Schulz's (1927) sectional classification (Table 2) in Roman numerals in the phylogenetic ITS tree (Fig. 2).

Fitch parsimony analysis with a subset of *Draba* taxa from all major groups recognized in the overall analysis resulted in 12 most parsimonious trees (MPTs) 208 steps in length, CI of 70% (autapomorphies excluded), and RI of 69.9%. The results of the bootstrap and decay analysis are added to the strict consensus tree shown in Figure 3.

The overall analysis (Fig. 2), as well as the anal-

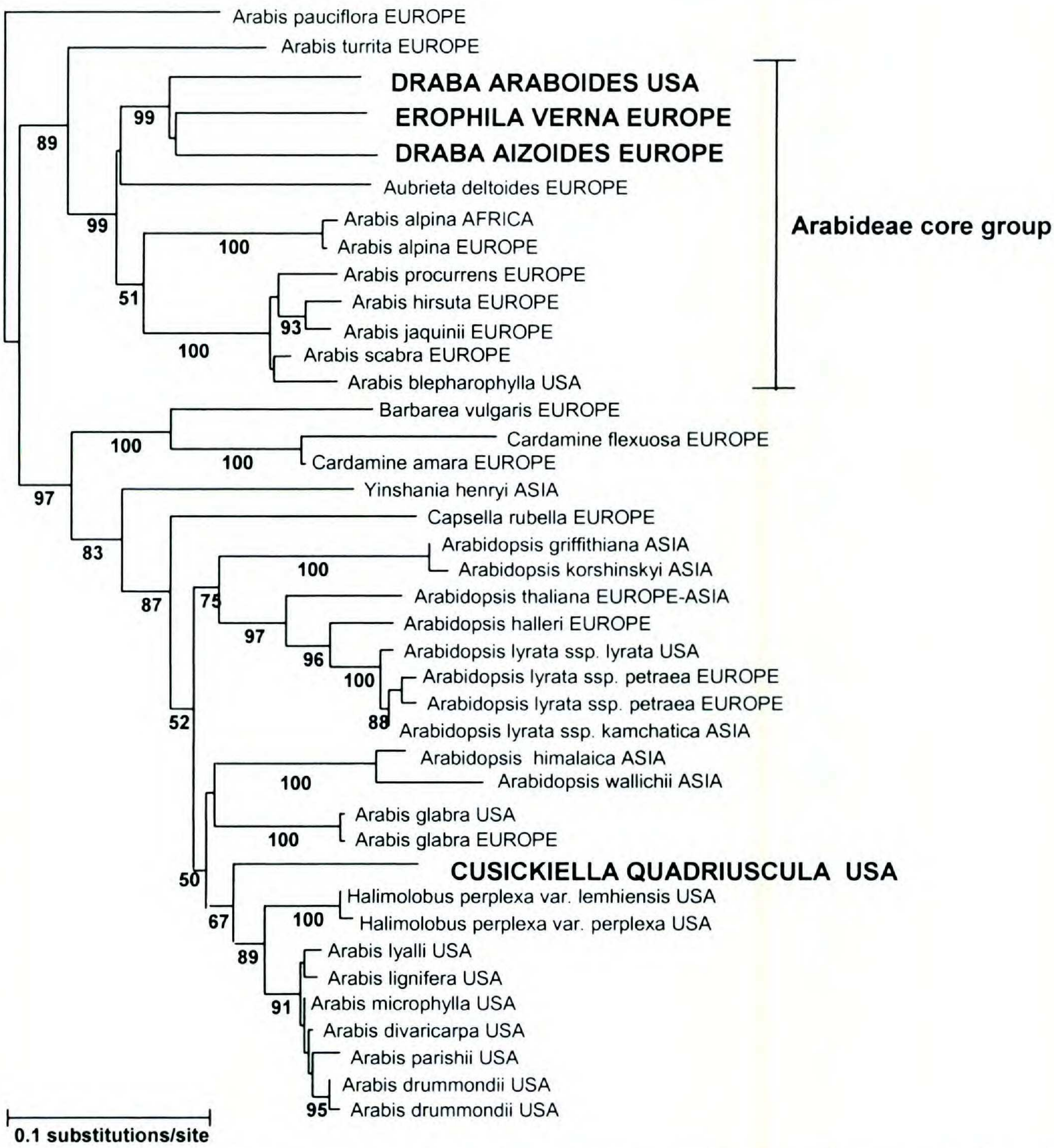

Figure 1. Neighbor-joining distance tree based on ITS sequence data to demonstrate the systematic relationships of *Draba* to other cruciferous taxa. Sequences from four taxa analyzed in this study (shown in capital letters) were added to a previous analysis of tribe Arabideae (Koch et al., 1999a). *Draba* and *Erophila* were integrated into an "Arabideae core group" previously recognized by Koch et al. (1999a). Distributional area is indicated. For *Arabis glabra* (L.) Bernh. herein we used the taxonomically more appropriate synonym *Turritis glabra* L. Bootstrap support is given below the branches from 1000 replicates.

ysis of a subset of taxa (Fig. 3), recognized the same six major ITS clades among European and American *Draba*, including the two accessions of *Erophila*. European *Draba* are confined to clade III together with European *Erophila* (Figs. 2 and 3); this clade is characterized by long internal genetic distances separating European taxa from the remaining American *Draba*. The American species of *Draba* are found in all remaining clades I, II, IV, V, and VI.

The most basal clade, clade I, combines sequences from mostly North American *Draba* taxa (*D. cuneifolia*, acc. no. 61; *D. platycarpa*, acc. no. 69; *D. reptans*, acc. no. 72), but also one accession from Peru (*D. araboides*, acc. no. 84). Clades I–III are well supported by bootstrap values of 100%, 88%, and 93%, respectively. In contrast, clades IV, V, and VI (combining the majority of taxa) are only weakly supported by bootstrap analysis (Fig. 2). However, all clades were recognized in the parsimony analysis with a subset of taxa from all six clades. The only exception is *D. scopulorum* (acc.

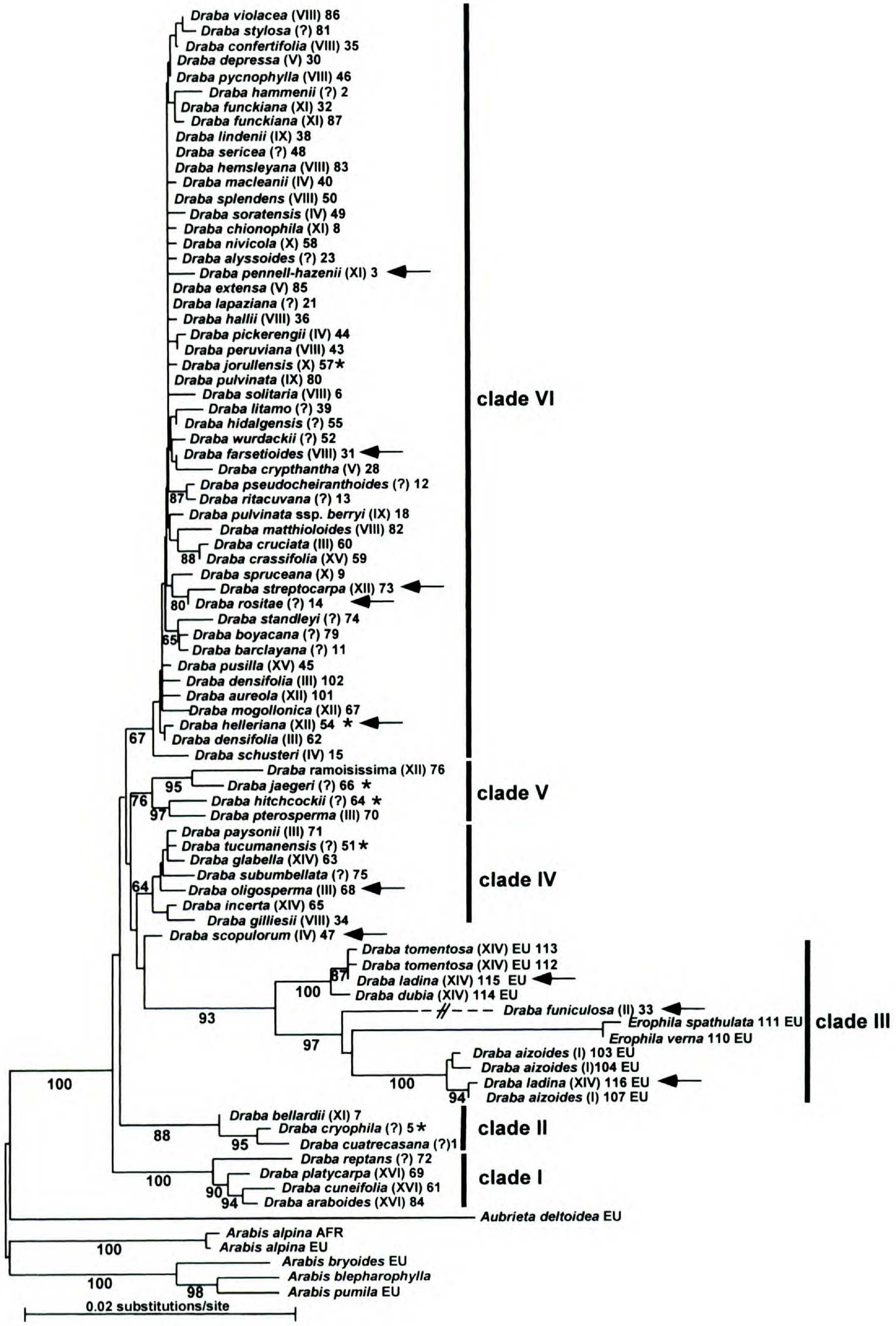
Draba violacea (VIII) 86
Draba stylosa (?) 81
Draba confertifolia (VIII) 35
Draba depressa (V) 30
Draba pycnophylla (VIII) 46
Draba hammenii (?) 2
Draba funckiana (XI) 32
Draba funckiana (XI) 87
Draba lindenii (IX) 38
Draba sericea (?) 48
Draba hemsleyana (VIII) 83
Draba macleanii (IV) 40
Draba splendens (VIII) 50
Draba soratensis (IV) 49
Draba chionophila (XI) 8
Draba nivicola (X) 58
Draba alyssoides (?) 23
Draba pennell-hazenii (XI) 3
Draba extensa (V) 85
Draba lapaziana (?) 21
Draba hallii (VIII) 36
Draba pickerengii (IV) 44
Draba peruviana (VIII) 43
Draba jorullensis (X) 57*
Draba pulvinata (IX) 80
Draba solitaria (VIII) 6
Draba litamo (?) 39
Draba hidalgensis (?) 55
Draba wurdackii (?) 52
Draba farsetioides (VIII) 31
Draba crypthantha (V) 28
Draba pseudocheiranthoides (?) 12
87
Draba ritacuvana (?) 13
Draba pulvinata ssp. berryi (IX) 18
Draba matthioloides (VIII) 82
Draba cruciata (III) 60
88
Draba crassifolia (XV) 59
Draba spruceana (X) 9
Draba streptocarpa (XII) 73
80
Draba rositae (?) 14
Draba standleyi (?) 74
65
Draba boyacana (?) 79
Draba barclayana (?) 11
Draba pusilla (XV) 45
Draba densifolia (III) 102
Draba aureola (XII) 101
Draba mogollonica (XII) 67
Draba helleriana (XII) 54 *
67
Draba densifolia (III) 62
Draba schusteri (IV) 15
Draba ramoisissima (XII) 76
95
Draba jaegeri (?) 66 *
76
Draba hitchcockii (?) 64 *
97
Draba pterosperma (III) 70
Draba paysonii (III) 71
Draba tucumanensis (?) 51*
Draba glabella (XIV) 63
Draba subumbellata (?) 75
64
Draba oligosperma (III) 68
Draba incerta (XIV) 65
Draba gilliesii (VIII) 34
Draba scopulorum (IV) 47
Draba tomentosa (XIV) EU 113
Draba tomentosa (XIV) EU 112
87
Draba ladina (XIV) 115 EU
100
Draba dubia (XIV) 114 EU
93
Draba funiculosa (II) 33
Erophila spathulata 111 EU
Erophila verna 110 EU
97
Draba aizoides (I) 103 EU
Draba aizoides (I)104 EU
100
Draba ladina (XIV) 116 EU
100
94
Draba aizoides (I) 107 EU
Draba bellardii (XI) 7
Draba cryophila (?) 5*
88
95
Draba cuatrecasana (?)1
Draba reptans (?) 72
Draba platycarpa (XVI) 69
100
Draba cuneifolia (XVI) 61
90
94
Draba araboides (XVI) 84
Aubrieta deltoidea EU
Arabis alpina AFR
100
Arabis alpina EU
Arabis bryoides EU
Arabis blepharophylla
100
98
Arabis pumila EU
0.02 substitutions/site
clade VI
clade V
clade IV
clade III
clade II
clade I

Table 2. Sectional classification system according to Schulz (1927). Roman numerals correspond to those used in parentheses in Figure 2.

Sectional classification according to Schulz (1927)	Number of species and distribution
I. *Aizopsis* DC.	28 spp., Mediterranean and rest of Europe
II. *Linodraba* O. E. Schulz	1 sp., South American (Falkland Islands and Patagonia)
III. *Chrysodraba* DC.	57 spp., Asia and North America
IV. *Rhabdodraba* O. E. Schulz	10 spp., South America
V. *Tylodraba* O. E. Schulz	8 spp., South America (Ecuador and Peru)
VI. *Acrodraba* O. E. Schulz	1 sp., NW Africa (Morocco)
VII. *Helicodraba* O. E. Schulz	2 spp., NW Africa (Morocco)
VIII. *Cladodraba* O. E. Schulz	14 spp., South America (Colombia south into Patagonia)
IX. *Dolichostylis* (Turcz.) O.E. Schulz	3 spp., South America (Venezuela)
X. *Adenodraba* O. E. Schulz	9 spp., Central America (Mexico and Guatemala) and South America (Colombia, Ecuador, and Bolivia)
XI. *Chamaegongyle* O. E. Schulz	6 spp., South America (Colombia and Venezuela)
XII. *Phyllodraba* O. E. Schulz	29 spp., North America, E. Asia, and Himalayas
XIII. *Nesodraba* Greene	1 sp., North America
XIV. *Leucodraba* DC.	59 spp., Europe, Asia, North America, and South America (2 spp.)
XV. *Drabella* DC.	23 spp., Asia, North America, and South America (2 spp.)
XVI. *Tomostima* (Raf.) O. E. Schulz	6 spp., North America and South America (2 spp.)
XVII. *Abdra* (Greene) O. E. Schulz	1 sp., North America

no. 47). This species was positioned between clades IV and III by distance analysis (Fig. 2) with higher sequence similarity to taxa from clade IV, but parsimony analysis positioned it in clade IV with high confidence (Fig. 3). We obtained no significant phylogenetic structuring among these six major clades, and the trees remained unresolved in this respect.

TRNL DATA

We considered 76 sequences of which 69 are novel reports herein. In total we considered 72 different taxa. The alignment of the *trn*L intron is 382 bp in length and contained 6 bp of the first exon of the *trn*L(UAA) gene at the 5′-end and 30 bp of the second exon at its 3′-end. The alignment of the *trn*L-F-intergenic spacer region is 404 bp in length and contained 5 bp of the second exon of the *trn*L(UAA) gene at its 5′-end and 15 bp of the *trn*F gene at the 3′-end. Both alignments were combined to a final alignment 786 bp in length consisting of the entire *trn*L intron as well as the second exon of the *trn*L gene and the complete *trn*L-F-intergenic spacer. A region of six base pairs between the intron and the spacer that showed no variation among *Draba* accessions sequenced by Widmer and Baltisberger (1999b) is missing. The whole data matrix is interspersed with 24 gaps (7 within the *trn*L intron, including 4 autapomorphies, and 17 within the spacer region, including 8 autapomorphies, respectively). Within the *trn*L intron 47 variable nucleotide positions were detected (including 20 autapomorphies); within the *trn*L-F spacer 52 out of 87 variable nucleotide positions were uninformative. The distance tree is shown in Figure 4 with support indicated by bootstrap greater than 50%.

Fitch parsimony analysis with a subset of taxa similar to the ITS analysis resulted in 182 MPTs with 112 steps in length, CI of 72%, and RI of 79%. This subset comprises the same set of taxa used for ITS analysis with additional *Draba rositae* (acc. no. 14), because this taxon has been separated from other *Draba* taxa by a relatively high bootstrap support of 63% (Fig. 4). Significant results of the bootstrap and decay analysis are shown along

←

Figure 2. Neighbor-joining distance tree of ITS sequence data. Bootstrap support is given from 1000 replicates. Arrows indicate positions of taxa that differ from the plastid analysis (see Fig. 4). Asterisks mark accessions that were used for triplet comparisons of ITS types to investigate concerted evolution (for details refer to text). Branch length of *Draba funiculosa* (acc. no. 33, clade III) has not been drawn to scale (refer to text). Taxonomic accession numbers correspond to those in Table 1. In parentheses the sectional position according to Schulz (1927) is given. Taxa that were not recognized by Schulz (1927) are marked with "?". ITS sequences of outgroup taxa from *Arabis* and *Aubrieta* have been published previously by Koch et al. (1999a). European *Draba* are indictaed by "EU."

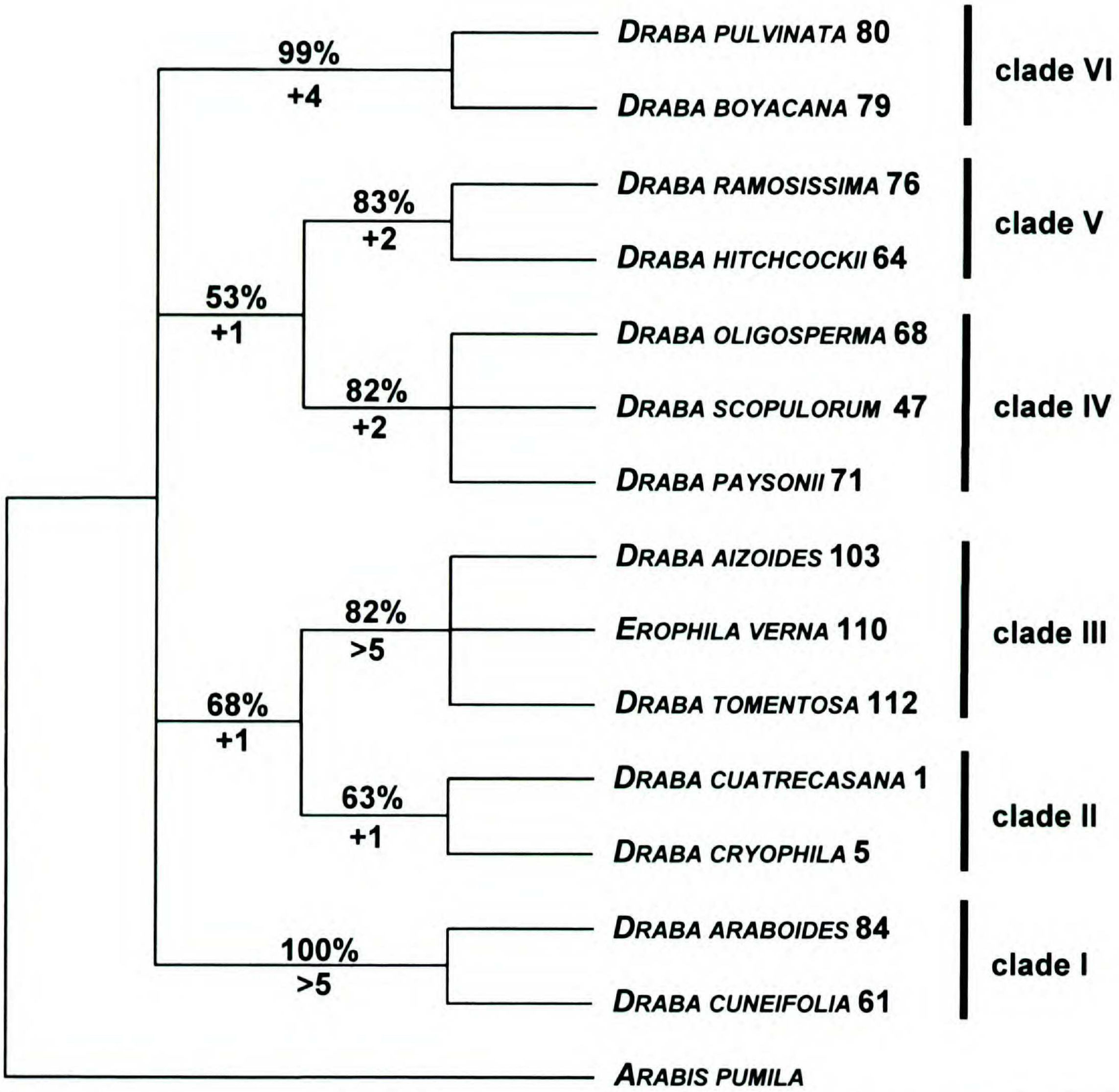

Figure 3. Strict consensus tree from Fitch parsimony using a subset of ITS sequences. Bootstrap support from 1000 replicates is seen above branches; decay values are indicated below branches. Accession numbers following taxa correspond to Table 1. Clades I–V correspond to ITS clades I–V from Figure 2.

branches in the strict consensus tree (Fig. 5). The results of this analysis are congruent with the findings of the overall distance analysis (Fig. 4). Five major clades could be distinguished (clades A–E, Figs. 4 and 5) with bootstrap support greater than 50%. There is no support to combine *D. oligosperma* (acc. no. 68) and *D. scopulorum* (acc. no. 47) to any of these clades. However, *D. oligosperma* is more closely related to cp genome types C and D (Figs. 4 and 5) as revealed from distance and parsimony analysis.

The occurrence of these particular cp genome types according to their geographic origin is summarized in Table 3.

The more inclusive distance analysis (Fig. 4) recognized five major clades among *Draba*, with *D. cuneifolia* (acc. no. 61), *D. platycarpa* (acc. no. 69), *D. reptans* (acc. no. 72), and *D. araboides* (acc. no. 84) in clade A only distantly related to the remaining clades. Plastome types from European *Draba* were found mostly in clades B and D, but one additional plastome type from *Draba aizoides* (acc. no. 108) (Widmer & Baltisberger, 1999b) showed an intermediate position between clades E and C/D. In the present analysis, three plastome types of *D. aizoides* from Widmer and Baltisberger (1999b) were included and all three were diverse. *Draba aizoides* herein investigated from Germany (acc. no. 103) is more closely related to *D. aizoides* (acc. no. 107 from Widmer & Baltisberger, 1999b); it assorts

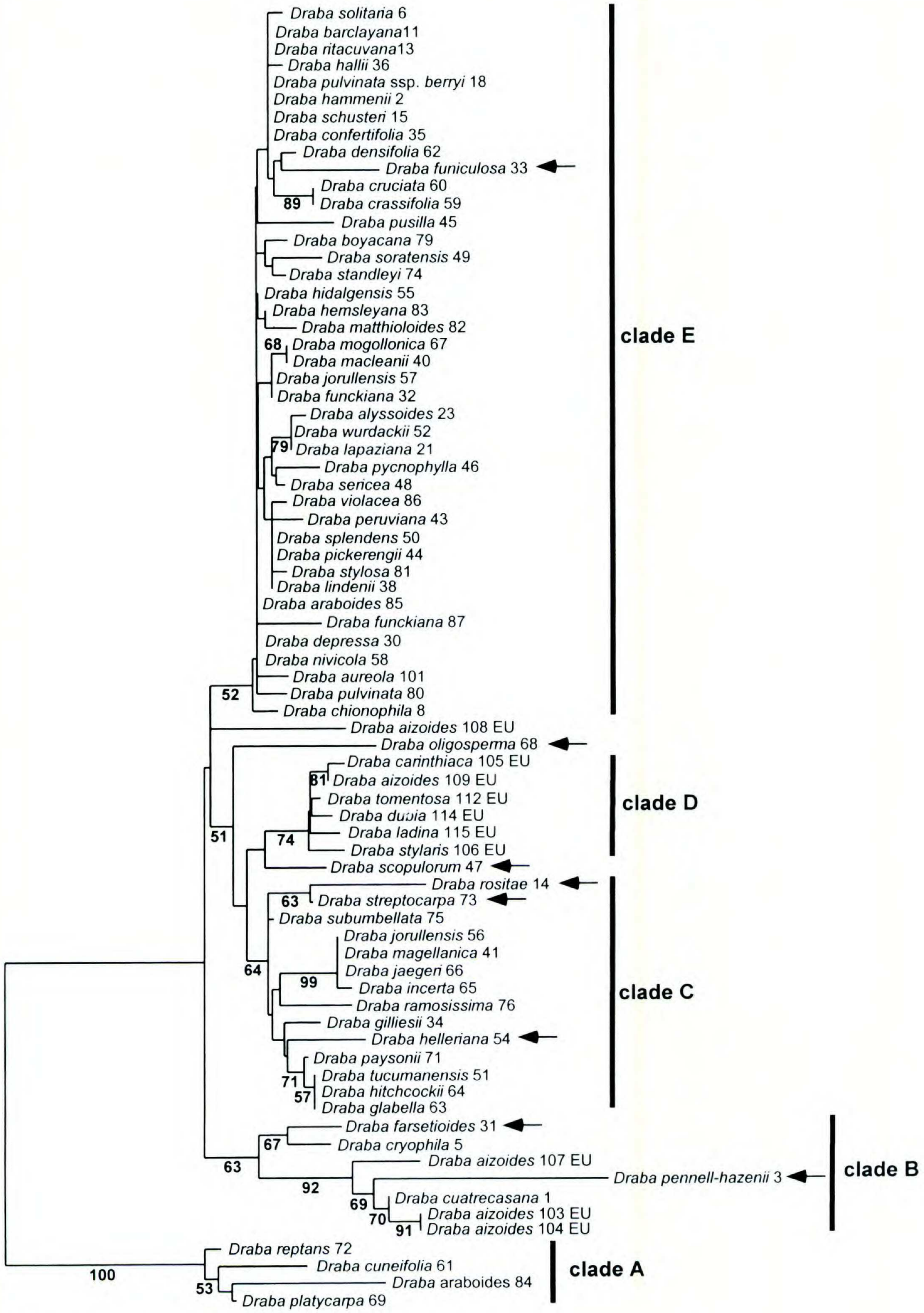

Figure 4. Neighbor-joining distance tree of *trn*L sequence data. Bootstrap support is given from 1000 replicates. Arrows indicate positions of taxa that differ from the ITS analysis (see Fig. 2). Accession numbers of taxa correspond to Table 1. European *Draba* are indictaed by "EU."

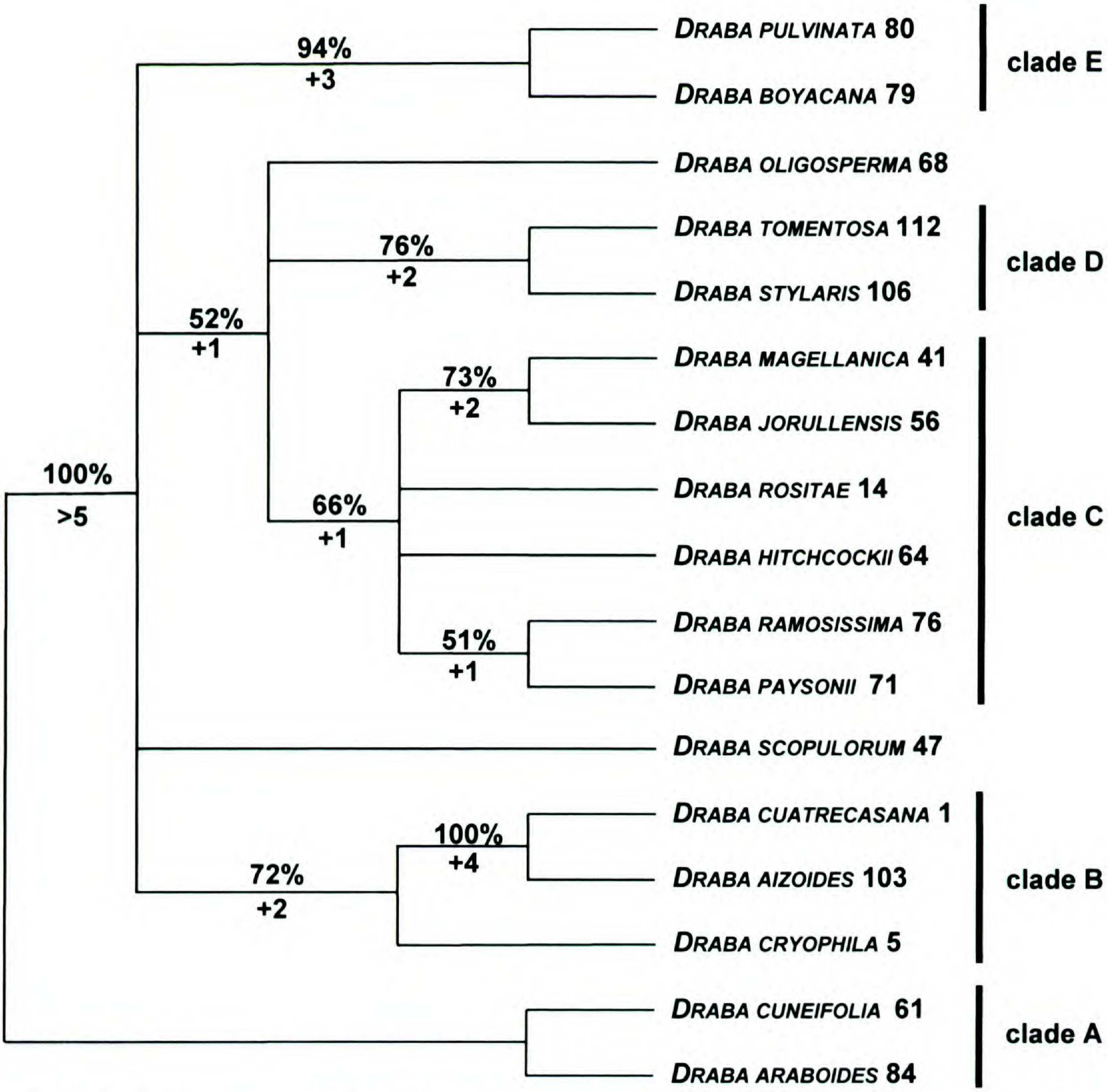

Figure 5. Strict consensus tree from Fitch parsimony using a subset of *trn*L sequences. Bootstrap support from 1000 replicates is seen above branches; decay values are indicated below branches. Accession numbers following taxa correspond to Table 1. Clades A–E correspond to *trn*L clades A–E from Figure 4.

within clade B (Fig. 4). These two plastomes of *D. aizoides* are separated by seven mutations. Some plastome types (*D. scopulorum*, acc. no. 47, *D. oligosperma*, acc. no. 68, and *D. aizoides*, acc. no. 107 from Widmer & Baltisberger, 1999b) are not supported significantly within any of the five clades A to E (Figs. 4 and 5). The plastome type of *Draba oligosperma* is positioned basal to clades C and D in distance and parsimony analyses (Figs. 4 and 5). The plastome type of *D. scopulorum* is supported within clade D by distance analysis (Fig. 4); however, this is not supported by a bootstrap value greater than 50%, and parsimony analysis with a reduced data set (Fig. 5) provided no information at all. cpDNA sequence data from *Erophila* were not available, and research of the authors focusing on annual Eurasian *Draba* is in progress.

ITS VERSUS *trn*L DATA

There is some congruence between phylogenetic trees from both data sets (Figs. 2 and 4): (1) American *D. cuneifolia* (acc. no. 61), *D. platycarpa* (acc. no. 69), *D. reptans* (acc. no. 72), and *D. araboides* (acc. no. 84) are separated from all remaining *Draba* taxa, American as well as European material, and are grouped in ITS clade I or corresponding *trn*L clade A; (2) the majority of American *Draba* is combined to ITS clade VI, which largely corresponds to *trn*L clade E; (3) taxa that were integrated

Table 3. Distribution of plastome types A–E among *Draba* accessions analyzed herein according to their sampling area. Unique or "ambiguous" plastome type of *D. scopulorum*, *D. oligosperma*, and *D. aizoides* (acc. nos. 47, 68, and 108, respectively) have been excluded here. Numbers of accessions showing a particular plastome type are indicated.

Origin of analyzed accessions	Plastome type (according to Fig. 2)				
	A	B	C	D	E
Europe		3		6	
U.S.A.	3		8		5
Peru	1				8
Venezuela		1			6
Colombia		3	1		5
Argentina			2		1
Chile			1		1
Mexico			2		3
Bolivia					2
Ecuador					10

into ITS clades IV and V possess a plastome type typical for taxa from clade C; and (4) European *Draba* is divided into two groups among both data sets (clade B and D in the plastid data set; and significant subgrouping within clade III in the ITS data set including *Erophila*), but the separation of taxa forming clades B and D does not correspond to that in the ITS clade III.

However, eight accessions (*D. pennell-hazenii*, acc. no. 3; *D. rositae*, acc. no. 14; *D. farsetioides*, acc. no. 31; *D. funiculosa*, acc. no. 33; *D. scopulorum*, acc. no. 47; *D. helleriana*, acc. no. 54; *D. oligosperma*, acc. no. 68; and *D. streptocarpa*, acc. no. 73) from America assign to different clades when both phylogenies (Figs. 2 and 4) were compared with each other. In addition, plastome types from *Draba aizoides* are found at three different positions (Fig. 4). Most American *Draba* species with ITS type VI (Fig. 2) possess a plastome type grouping into clade E (Fig. 4). However, American *Draba rositae* (acc. no. 14), *D. helleriana* (acc. no. 54), and *D. streptocarpa* (acc. no. 73) from ITS clade VI do have the plastome type C and not type E, as in the majority of taxa investigated, and within *D. pennell-hazenii* (acc. no. 3) and *D. farsetioides* (acc. no. 31) from ITS clade VI we found a plastome type from clade B. *Draba oligosperma* (acc. no. 68) from ITS clade IV (Fig. 2) and *D. scopulorum* (acc. no. 47), which is also grouped to ITS clade IV in parsimony analysis (Fig. 3), do not have the plastome type C (Fig. 4) as do the remaining taxa of ITS clade IV. However, both plastomes of these taxa, *D. scopulorum* and *D. oligosperma*, are found peripheral to plastome clade C (Fig. 4). Interestingly, American *D. funiculosa* (acc. no. 33), with an ITS type closer to European *Draba*, showed a plastome type grouping to clade E (Fig. 4).

Discussion

Status of Tribe Alysseae and the Phylogenetic Position of Genus *Draba*

Preliminary molecular phylogenetic analysis (Koch, unpublished) suggests that the morphologically poorly defined tribe Alysseae is not monophyletic. Two of Schulz's (1936) tribes, Lunarieae and Drabeae, were recognized by Janchen (1942) and Hayek (1911) as subtribes of the Alysseae. The genus *Camelina*, which was treated by Al-Shehbaz (1987) in the Alysseae, was shown by Zunk et al. (1999) to be very close to genus *Capsella*, a genus traditionally placed in the tribe Lepidieae. A majority of the 650 species of the Alysseae (sensu Janchen, Hayek, and Al-Shehbaz) belong to the genera *Alyssum* (170 spp.) and *Draba* (350 spp.). The present analysis supports a closer relationship among *Draba*, Eurasian *Arabis*, and the European *Aubrieta*. Eurasian *Arabis* and *Aubrieta* have been shown to represent a core group of the tribe Arabideae (Koch et al., 1999a). However, as traditionally delimited by Janchen (1942), Hayek (1911), or Schulz (1936), the tribe Arabideae breaks down as an unnatural group (Koch et al., 1999a) (Fig. 1). Similarily, the tribe Lepideae is not monophyletic, and several of its constituent genera are sister taxa in other tribes of the Brassicaceae (Koch et al., 2000, 2001). The consistent conclusion reached from all molecular studies of the Brassicaceae is that the tribal relationships of Schulz (1936), Janchen (1942), and Hayek (1911), which are based solely on morphological characters, are highly artificial and do not reflect phylogenetic relationships. Such artificiality was also elucidated at the generic level, especially by molecular studies on *Cochlearia* (Koch et al., 1999b), *Thlaspi* (Mummenhoff et al., 1997a, b), and *Arabis* (Koch et al., 1999a, 2000, 2001). Our findings should encourage other investigators of cruciferous genera to test phylogenetic assumptions at a higher taxonomic level.

Hybrid ITS Types and Reticulation

We found no ambiguous nucleotide positions when sequencing *Draba* ITS regions, although hybrid origin for several taxa is likely because of some characteristics frequently found in species groups showing hybridization and introgression: (1) incongruencies between nuclear- and plastid-de-

Table 4. References for chromosome numbers among *Draba* taxa under study. For taxa that were recognized by Schulz (1927) information about his sectional treatment is given (see also Table 2).

Sectional classification according to Schulz (1927)	Taxon	Chromosome no. and reference	Ploidy level
Not mentioned by Schulz	*D. cryophila*	$2n = 48$ (Galland & Pfitsch, 1986b)	hexaploid
Adenodraba	*D. jorullensis*	$n = 12$ (Beaman et al., 1962)	triploid
Aizopsis	*D. aizoides*	$2n = 16$ (Hess et al., 1977)	diploid
Calodraba	*D. gilliesii*	$n = 24$ (Boecher, 1966)	hexaploid
Chamaegongyle	*D. bellardii*	$2n = 48$ (Galland & Pfitsch, 1986a)	hexaploid
	D. chionophila	$2n = 48$ (Galland & Pfitsch, 1986a)	hexaploid
Chrysodraba	*D. oligosperma*	$2n = 32, 64$ (Mulligan, 1972)	tetraploid, octoploid
	D. paysonii	$2n = 42$ (Mulligan, 1971)	pentaploid + 2
Doliostylis	*D. pulvinata*	$2n = 48$ (Galland & Pfitsch, 1986a)	hexaploid
Drabella	*D. crassifolia*	$2n = 40$ (Price, 1979)	pentaploid
Leucodraba	*D. carinthiaca*	$2n = 16$ (Hess et al., 1977)	diploid
	D. dubia	$2n = 16$ (Hess et al., 1977)	diploid
	D. fladniziensis.	$2n = 16$ (Hess et al., 1977)	diploid
	D. glabella	$n = 32, 40$ (Mulligan, 1970)	octoploid, decaploid
	D. incerta	$n = 56$ (Mulligan, 1966)	14-ploid
	D. ladina	$2n = 32$ (Hess et al., 1977)	tetraploid
	D. magellanica	$n = 32$ (Heilborn, 1941)	octoploid
	D. stylaris	$2n = 32$ (Hess et al., 1977)	tetraploid
	D. tomentosa	$2n = 16$ (Hess et al., 1977)	diploid
Phyllodraba	*D. helleriana*	$n = 8$ (Ward, 1983), $n = 9$ (Ward & Spellenberg, 1988)	diploid
	D. mogollonica	$n = 16$ (Ward, 1983)	tetraploid
	D. ramosissima	$n = 8$ (Nye, 1969)	diploid
	D. streptocarpa	$n = 20$ (Price, 1979)	pentaploid
Rhabdodraba	*D. pickerengii*	$2n = 24$ (Favarger & Huynh, 1965)	triploid
Tomostima	*D. cuneifolia*	$2n = 16$ (Rollins & Ruedenberg, 1971)	diploid
	D. platycarpa	$2n = 32$ (Rollins & Ruedenberg, 1971)	tetraploid
	D. reptans	$2n = 16$ (Löve & Löve, 1982)	diploid, tetraploid
		$2n = 32$ (Smith, 1965)	

rived phylogenetic hypotheses, (2) the occurrence of polyploids (Table 2). Additionally, for European *Draba* extensive hybridization and reticulation have been shown previously (Widmer & Baltisberger, 1999a, b). Therefore, we have to assume that concerted evolution of putative ancestral ITS types resulted in one dominant ITS copy. Different modes of concerted evolution of ITS regions have been discussed, e.g., for *Gossypium* (Wendel et al., 1995a, b), roses (Vissemann, 2000), *Quercus* (Muir et al., 2001), and other plants (Buckler et al., 1997), and for some cruciferous taxa (*Hilliella, Cochleariella* in Koch & Al-Shehbaz, 2000; *Aradidopsis* in O'Kane et al., 1996; *Cardamine* in Franzke et al., 1998; *Microthlaspi* in Mummenhoff et al., 1997b). Sequence divergence values of ITS types from putative parents, which produced hybrids in which concerted evolution has been documented, range from 3.1 to 7.8% (Koch & Al-Shehbaz, 2000: 270). Sequence divergence values for *Draba* ITS types exceeded 9%, and these might indicate a relatively old age for the genus. Divergence time estimates of cruciferous plants are found in Koch et al. (2000) for chalcone synthase and alcoholdehydrogenase and in Koch et al. (2001) for chalcone synthase and maturase K. A comparison of these time estimates with an ITS-derived phylogeny comprising a similar set of species (Koch et al., 1999a) shows that 1% ITS sequence divergence correspond to approximately 0.5 to 1.0 million years.

American *Draba* occur at different phylogenetic positions, sometimes together with European *Draba* plastome types (Fig. 4). They include numerous polyploids (Table 4), and it is likely that hybridization and polyploidization played a major role in their evolution. In order to test this assumption and show interrelationships between different ITS types, several comparisons of three ITS sequences from three different ITS clades (Fig. 2) were done to analyze the distribution of variable nucleotides. These

American taxa compared as such are represented by asterisks in Figure 2. First, American *Draba cryophila* (acc. no. 5, clade II) was compared with *D. hitchcockii* (acc. no. 64) and *D. tucumanensis* (acc. no. 51) of clades V and VI, respectively. The distribution of nucleotide diversity revealed that *D. hitchcockii* had 21 characters in common with *D. tucumanensis* and only 5 characters with *D. cryophila* (acc. no. 5). Of the eight nucleotide positions where *D. hitchcockii* differed from *D. cryophila* and *D. tucumanensis*, three were identical to positions from *D. jaegeri* (acc. no. 66, also from clade V). Synapomorphic characters were found within the ITS2 regions of *D. tucumanensis* (acc. no. 51) and *D. hitchcockii* (acc. no. 64), but not within *D. cryophila* (acc. no. 5) and *D. hitchcockii* (acc. no. 64) or *D. jaegeri* (acc. no. 66).

Different results were obtained upon comparison of *D. jorullensis* (acc. no. 57) of clade VI (Fig. 2) with *D. tucumanensis* (acc. no. 51) and *D. hitchcockii/D. jaegeri* (accession nos. 64 and 66). In this case, both *D. hitchcockii* and *D. jaegeri* shared five characters with *D. jorullensis* (acc. no. 57) and seven with *D. tucumanensis* (acc. no. 51). Three of eight characters of *D. hitchcockii* (acc. no. 64), which were not found in *D. tucumanensis* (acc. no. 51) and *D. jorullensis* (acc. no. 57), were synapomorphic with those of *D. jaegeri* (acc. no. 66). Furthermore, *D. tucumanensis* (acc. no. 51) contributed to all variable nucleotide positions in the ITS2 region shared with either *D. hitchcockii* (acc. no. 64) or *D. jaegeri* (acc. no. 66), whereas *D. jorullensis* (acc. no. 57) contributed nucleotide positions only within the ITS1 region. These findings are interpreted as the result of hybridization between taxa of clades IV and VI, as represented by *D. tucumanensis* and *D. jorullensis*, respectively, which produced the hybrid ITS types found in clade V as represented by *D. hitchcockii* and *D. jaegeri* (Fig. 2).

Four additional comparisons are made to test the possible role of hybridization in the formation of ITS types of clade VI (Fig. 2) that includes no European taxa: (a) *D. cryophila* (acc. no. 5, clade II), *D. jorullensis* (acc. no. 57, clade VI), and *D. tucumanensis* (acc. no. 51, clade IV); (b) *D. cryophila* and *D. helleriana* (acc. nos. 5 and 54, basal in clade VI), and *D. tucumanensis* (acc. no. 51, clade IV); (c) *D. tucumanensis* and *D. jorullensis* (acc. nos. 51 and 57) and *D. helleriana* (acc. no. 54); as well as (d) *D. cryophila*, *D. helleriana*, and *D. jorullensis* (acc. nos. 5, 54, and 57, respectively).

The first two comparisons (first, among acc. nos. 5, 51, 57, and second, among 5, 51, 54) revealed that accessions 54 and 57 had only 6 characters in common with accession number 5 of clade II but 23 characters with accession number 51 of clade IV. The third comparison (acc. nos. 51, 54, and 57) revealed that all synapomorphic characters combined exclusively accession numbers 54 and 57. This might indicate close relationships of taxa from clade VI, which includes accession numbers 54 and 57, and a monophyletic origin. The fourth comparison (acc. nos. 5, 54, and 57) provides no additional data toward the hybridization of taxa from either clade II or IV as a source for ITS types found in clade VI. Accession number 54 shared only 3 characters with accession number 5, but accession number 54 shared 21 characters with accession number 57. Therefore, based on ITS sequences, it was not possible to verify the hypothesis that ITS types from clade VI evolved via concerted evolution after hybridization.

Comparison of the maternal plastid phylogeny (see taxa indicated by black arrows, Fig. 4) with the ITS-derived phylogeny indicates that plastome types found in *D. pennell-hazenii* (acc. no. 3) and *D. farsetioides* (acc. no. 31) similar to those found in European taxa (plastid clade B) are distributed among accessions with an ITS type of clade VI. By contrast, *D. funiculosa* (acc. no. 33) has a plastome type found in clade E, but its ITS copy is related to European ITS types found in ITS clade III. Plastome types from plastidic clade C (*D. rositae*, *D. helleriana*, and *D. streptocarpa* or acc. nos. 14, 54, 73, respectively) contributed to the genetic constitution of taxa found in ITS clade VI. Interestingly, plastome type E, which is distributed among American *Draba* of the ITS clade VI, is similar to a plastome type from the European *D. aizoides* (acc. no. 108, Fig. 4) but not from the other accessions of *D. aizoides* (acc. nos. 107, 109, 103), which assort to plastome clades B and D. Extensive intraspecific cpDNA haplotype variation in alpine *D. aizoides* was demonstrated by Widmer and Baltisberger (1999b). Therefore, one could speculate that ancient gene flow and chloroplast capture might have resulted in the similar transmission of European plastome types into American *Draba* and in the constitution of the whole complex of taxa with an ITS type from clade VI. Plastome types found in taxa from this group mostly belong to plastome type E (only 5 out of 51 accessions with ITS type VI have been shown to possess other plastome types than plastome type E) and are only weakly differentiated from each other. There is no resolution within clade E in the *trn*L-derived phylogeny.

Taking both approaches into account, the comparison of ITS types and the distribution of plastome types among different clades reveals strong

evidence for extensive reticulation during the evolution of the American complex of *Draba*. However, concerted evolution of ITS types has resulted in homogeneous ITS copies within single taxa: no difficulties were encountered while obtaining the sequences by direct cycle sequencing of the purified PCR products. The genetic source of plastome and ITS type variation probably originated from Eurasian taxa, for which very complex evolutionary scenarios have already been described. Subsequent hybridization probably took place among different groups of American *Draba* (e.g., taxa from ITS clade II with taxa from ITS clade IV resulted in taxa from ITS clade V), and some taxa from the ITS clade VI crossed back with taxa from clades V or IV possessing plastomes of type C. Only taxa from ITS clade I (corresponding to *trn*L clade A), all of which are distributed in America, represent a genetically separated lineage (Figs. 2, 4).

These hypotheses might be simplified; however, they will become more complex when additional individuals from a single taxon or one population are analyzed. For example, extensive ongoing gene flow has been reported within *Draba* from the Alps and Scandinavia (Widmer & Baltisberger, 1999a, b; Brochmann et al., 1992a–d; Brochmann et al., 1993). Although the study of herbarium material is greatly affected by undersampling, it is rather remarkable that in 8 out of the 74 samples of American *Draba* (> 10%) analyzed, incongruencies between ITS- and *trn*L-derived phylogenies were detected. One has to assume that much higher levels of incongruencies will be observed in genetic analyses at the populational level.

SECTIONAL CLASSIFICATION OF *DRABA*

Draba is generally recognized as a natural genus of about 350 species distributed primarily in the Arctic, subarctic, and alpine regions of the Northern Hemisphere, with about 65 species in South America along the Andes from Colombia to Patagonia (Al-Shehbaz, 1987). In the present study we focused on American *Draba*, including a limited number of species from Europe (8 species from 10 accessions). Eleven species grow in Mexico and Central America (Rollins, 1984). More than 100 grow in North America and Greenland (Rollins, 1993), with the ranges of about 20 of these extending into the Arctic and subarctic Eurasia.

Schulz (1927, 1936) divided *Draba* (excluding *Erophila*) into 17 sections (Table 2) that have been considered by some (e.g., Fernald, 1934; Al-Shehbaz, 1987) to be highly artificial. Schulz defined his sections primarily on the presence or absence of stem leaves, flower color (white vs. yellow), the presence or absence of median nectaries, and style length. Four of Schulz's sections are no longer recognized in *Draba*. Section *Helicodraba* O. E. Schulz was shown by Hyam and Jury (1990) to belong to the Southwest Asian *Graellsia* Boiss. Section *Nesodraba* (Greene) N. Busch was reduced by Berkutenko (1995) to *Schivereckia* Andrzeiowski ex DC. Sections *Tomostima* (Raf.) O. E. Schulz and *Abdra* (Greene) O. E. Schulz are believed to form the well-defined genus *Tomostima* Raf. (Price & Al-Shehbaz, unpublished). Species as assigned to the remaining sections are still maintained in the genus and are not currently questioned (Table 2). Section *Acrodraba* O. E. Schulz (1 species, *Draba oreadum* Maire) is restricted to northern Africa, whereas sections *Rhabdodraba* O. E. Schulz (10 species), *Adenodraba* O. E. Schulz (9 species), *Tylodraba* O. E. Schulz (8 species), *Dolichostylis* (Turcz.) O. E. Schulz (3 species), *Calodraba* O. E. Schulz (14 species), and *Chamaegongyle* O. E. Schulz (6 species) are distributed in Central and South America. With the exception of section *Aizopsis* DC. (28 species), which is almost exclusively European, larger sections include the North American and Eurasian *Chrysodraba* DC. (57 species), the North American and East Asian *Phyllodraba* O. E. Schulz (29 species), European and central Asian *Leucodraba* DC. (59 spp.), and the Asian *Drabella* DC. (23 spp.). The last included two South American species that doubtfully belong there.

Four out of the 17 sections sensu Schulz (1927), which comprise only five species (1.5% of the total in *Draba*), were not included in the present study. Sections *Helicodraba* and *Nesodraba* (4 species) have already been combined under different genera as outlined above. Material of the North American section *Abdra* (1 species) and African section *Acrodraba* (1 species) were not available. Schulz's sectional classification is given in Roman numerals in parentheses in the phylogenetic ITS tree (Fig. 2).

The phylogenetic analysis of the ITS sequence data (Fig. 2) shows that all sections exclusively distributed in South and Central America (Table 2, sects. *Rhabodraba, Tylodraba, Calodraba, Dolichostylis, Adenodraba, Chamaegongyle*) fall within clade VI. This clade also includes such taxa as *Draba aureola* (acc. no. 101) of section *Phyllodraba* (North America, Asia), *D. crassifolia* (acc. no. 59) from section *Drabella* (Eurasia, North America), and *D. cruciata* (acc. no. 60) of section *Chrysodraba* (Eurasia, North America). This suggests that South and Central American *Draba* probably evolved from other sections distributed in the Northern Hemisphere. With our molecular data it is difficult

to distinguish possible, relatively recent South-North migration events (from South America to the North) from the earlier North-South migration route. However, in this case some morphologically defined species should have a much wider distribution range, which is not the case.

A few species from exclusively South and Central American sections fall within other clades. For example, *Draba bellardii* (acc. no. 7) of section *Chamaegongyle* belongs to the ITS clade II, whereas both *D. scopulorum* (acc. no. 47) of section *Rhabdodraba* and *D. gilliesii* (acc. no. 34) of section *Calodraba* belong to clade IV.

Some level of congruence was found when comparing the ITS clustering of European *Draba* with the sectional classification of Schulz (1927, 1936). Taxa comprising the ITS clade III were assigned to sections *Aizopsis*, e.g., *D. aizoides*, and *Leucodraba*, e.g., *D. tomentosa*. These sections are primarily Eurasian. Interestingly, *D. funiculosa* (acc. no. 33) falls within this ITS clade, but the extremely long branch (more than twice longer than the maximum distance found within the total ingroup) that sets it off from the remaining European taxa indicates that its ITS type has considerably diverged. This particular subantarctic species comprises the monotypic section *Linodraba*.

A comparison of Schulz's (1927, 1936) sectional classification with the present molecular study reveals that section *Tomostima* (XVI, Table 2), which corresponds to the ITS clade I (Fig. 2) and *trn*L clade A (Fig. 4), is the most clearly supported.

Schulz (1927, 1936) and all European taxonomic treatments maintained *Erophila* as a distinct genus that is closely related to *Draba*. However, the present ITS data show that it is better integrated into *Draba*, and these results support the view held by most North American botanists (e.g., Fernald, 1934; Al-Shehbaz, 1987; Rollins, 1993). The only morphological difference between *Erophila* and *Draba* is the presence of bifid petals in *Erophila* rather than entire to deeply lobed ones in *Draba* (Al-Shehbaz, 1987).

In conclusion, our findings do not provide much support for Schulz's (1927, 1936) sectional classification, although they suggest some species tentatively correspond to a few of his sections. However, several taxa (*D. pennell-hazenii*, *D. rositae*, *D. farsetioides*, *D. funiculosa*, *D. scopulorum*, *D. helleriana*, *D. oligosperma*, and *D. streptocarpa*, acc. nos. 3, 14, 31, 33, 47, 54, 68, 73, respectively), which fall into different clades when the ITS- and *trn*L-derived phylogenies were compared, indicate extensive cross relationships not only among taxa of primarily South and Central American sections, but also among sections with other geographical centers of distribution, e.g., North America, Europe (Eurasia), and the subantarctic. A case in point is the South American *Draba magellanica* from Argentina (sect. *Leucodraba*, XIV), which has a chloroplast type (clade C, Fig. 4) not found within the remaining species of this section (e.g., *D. dubia* within *trn*L clade D). Two other species from Argentina (*D. funiculosa*, acc. no. 33, and *D. magellanica*, acc. no. 41) have chloroplast types E and C, respectively.

Although one might argue that in order to achieve a better understanding of the sectional classification within *Draba*, more analyses are needed, especially from the sections not included in the present study, it is safe to conclude that our molecular data clearly show that the conventional sectional classification of *Draba* as conceived by Schulz (1927, 1936) is an artificial one.

CHROMOSOME NUMBER, POLYPLOIDY, AND APOMIXIS

Because the present study is based on herbarium material, no chromosome counts were made from the plants investigated. However, several chromosome counts are available from the literature (Table 4). From this, it seems likely that the base chromosome number for *Draba* is $x = 8$. This is also the base chromosome number for sister European genera *Arabis* and *Aubrieta* (Fig. 1). Chromosome data demonstrate the existence of diploid, triploid, tetraploid, pentaploid, hexaploid, octoploid, and decaploid taxa based on $x = 8$ (Table 4). Higher chromosome numbers ($2n = 112$) have been reported for *D. incerta* (acc. no. 65) from North America (sect. *Leucodraba*).

The well-defined clade I (ITS data), or corresponding clade A (*trn*L data), comprising *D. cuneifolia* (diploid, acc. no. 61), *D. platycarpa* (tetraploid, acc. no. 69), *D. reptans* (diploid and tetraploid, acc. no. 72), and *D. araboides* (no count for chromosome number, acc. no. 84), included diploid and tetraploid species (Table 4). In summary, this clade (well defined as sect. *Tomostima*, XVI) comprises mostly diploid species (as known so far), and also represents an ancestral clade to all remaining *Draba*. This finding will need further investigation because section *Tomostima* is restricted to North and South America, and it is hard to believe that these taxa served as ancestors for Eurasian *Draba*. The consequence is the recognition of genus *Tomostima* (Price & Al-Shehbaz, unpublished).

The ITS data indicate a hybrid origin for *Draba*

ramosissima (clade V, acc. no. 76, Fig. 2), a species reported to be diploid (Table 4). As outlined before, direct ITS sequence comparisons demonstrated that ITS types from clade V most likely evolved from ancestral ITS types of clades VI and IV. Therefore, in *D. ramosissima* speciation via hybridization must have taken place at the diploid level and only diploid parental taxa could have served as progenitors. Another diploid, *D. helleriana* (acc. no. 54) has an ITS type corresponding to clade III (Figs. 2, 3, 6) and a plastome type to clade C (Figs. 4–6). This incongruency could be best explained by gene flow between different groups of taxa, thus indicating another hybrid speciation at the diploid level. Except for *D. cuneifolia* and *D. reptans* from section *Tomostima* and *D. helleriana* and *D. ramosissima* from section *Phyllodraba*, counts for the remaining American *Draba* species showed polyploidy (Table 4). By contrast, triploids (*D. jorullensis*, acc. no. 56; *D. pickerengii*, acc. no. 44), pentaploids (*D. crassifolia*, acc. no. 59; *D. streptocarpa*, acc. no. 73), and an aneuploid (*D. paysonii*, pentaploid + 2 chromosomes, acc. no. 71) have been reported (Table 4), and these taxa grouped to plastidic clades C and D or ITS clades IV and VI.

Apomixis has been documented in a few species such as *Draba verna* or *D. oligosperma* (Mulligan & Findlay, 1970; Mulligan, 1971, 1972, 1976; Price, 1980), and it is likely that it might be more widespread among American *Draba*. Therefore, in *Draba* apomixis might be an additional mechanism that led to the recognition of several hundred, morphologically defined taxa. The occurrence of apomixis has also been documented for other Brassicaceae, and North American *Arabis* parallels the situation in *Draba* (Böcher, 1966; Rollins, 1983). North American *Arabis*, from which many species were more appropriately transferred to *Boechera* (Löve & Löve, 1975; Weber, 1982, 1989), is a young taxon, presumably of Pleistocene origin. This genus exhibits remarkable morphological, ecological, and physiological diversification leading to the recognition of more than 50 species, and most of them show the trait apomixis.

As suggested by the low sequence divergence values, American *Draba* either of the ITS clade VI (Fig. 2) or the corresponding *trn*L clade E (Fig. 4) probably are relatively young taxa similar to *Boechera*. Comparable low levels of molecular variation have been found separating the North and South American *Noccaea* Moench, sensu Meyer (1973, 1979), or American *Thlaspi* L. sensu lato from their Eurasian relatives (Koch et al., 1993; Koch et al., 1998c). Contrary to this, in Europe several plastome types from *D. aizoides* were found by Widmer and Baltisberger (1999b), some of them very close to *D. aizoides*, accession number 107 (DA2, DA3, DA9, DA6, DA8, Widmer & Baltisberger, 1999b), separated by a maximum of seven mutations (in this analysis corresponding to clade B, Fig. 5), and one plastome type (DA7) was separated from *Draba aizoides*, acc. no. 108, by two mutations. All samples analyzed by Widmer and Baltisberger (1999b) originated from the Swiss Alps and demonstrate the enormous plastome variation even within one taxon (*Draba aizoides*) from a restricted area. ITS sequence variation among *D. aizoides* accessions (Fig. 2) is much lower, and all *D. aizoides* accessions analyzed herein confined to one group within ITS clade III. In this group we also found one *D. ladina* (acc. no. 116); this is not unexpected, because this taxon evolved by hybrid speciation involving *D. aizoides* as one parental taxon (Widmer & Baltisberger, 1999a).

The close relationships between the floras of Central Asia and western North America are well documented (Parks & Wendel, 1993, and references therein). The putative Bering bridge connected Asia and North America several times throughout the late Tertiary and Pleistocene (Parrish, 1987), and this land bridge probably served as an immigration route for several Brassicaceae, including *Thlaspi* (Payson, 1926) and *Stroganowia* Karelin & Kirilov (Rollins, 1982). Palynological analyses from the Venezuelan Andes document the occurrence of *Draba* in Holocene deposits after late Pleistocene deglaciation events (Salgado Labouriau et al., 1988).

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